REMARKS

Claims 105, 106, 109-131, 133-142, 144-164, 167-171, 184, and 185 are pending in this application.

35 U.S.C. § 112, Second Paragraph

The Office Action alleges that claims 141 and 142 are indefinite, stating at page 2:

> Claims 141 and 142 depend from claim 139 drawn to a method of use of a class I mannosidase inhibitor. By definition, class I mannosidase inhibitor inhibits mannosidase I. Mannosidase I is an enzyme that cleaves α1,2 linkages not α1,3 linkages or α1,6 linkages. Thus, class I mannosidase inhibitor prevents the removal of α1,2 mannose residues as a result of mannosidase I substrate specificity. Claims 141 and 142 recite the limitation "wherein the mannosidase inhibitor further prevents the removal of one" "α1,3 mannose residue" or "α1,6 mannose residue", respectively. While some specific mannosidase I inhibitors such as kifunensine and 1-deoxymannojirimycin may in addition weakly inhibit mannosidase II and consequently prevent the removal of α1,3 mannose residues or α1,6 mannose residue, the claims are not limited to said specific inhibitors (Shah et al. Biochemistry, 2003, Vol. 42, pages 13812-13816). Therefore, it is unclear how the inhibitor of mannosidase I can prevent removal of residues on which mannosidase I does not act. (emphasis added).

In claim 139, a class I inhibitor is recited. As the Office Action states, a class I mannosidase inhibitor can inhibit mannosidase I and prevent removal of α1,2 mannose residues. Claims 141 and 142 which depend from claim 139 narrow the subject matter of claim 139 by requiring that the class I inhibitor also be able to inhibit the removal of an α1,3 or α1,6 mannose residue, respectively. As the Office Action also states, some class I inhibitors also inhibit class II mannosidases (and thus prevent removal of α1,3 and/or α1,6 mannose residues). Claims 141 and 142 place no limitations on how well the class I inhibitor inhibits the removal of α1,3 or α1,6 mannose residues, thus, the fact that the inhibitor may do so "weakly" does not render the claim indefinite. In addition, Applicants do not know of a reason as to why these claims would have to be limited to two specific examples of class I inhibitors (e.g., kifunensine and 1-

deoxymannojirimycin) that can also inhibit class II mannosidases. Applicants submit that claims 141 and 142 properly depend from claim 139 and are definite, and request that this rejection be withdrawn.

35 U.S.C. § 103

Claims 105, 106, 109-128, 130, 131, 133-138, 184, and 185

The Office Action maintains the rejection of claims 105, 106, 109-128, 130, 131, 133-138, 184, and 185 as obvious in light of Friedman et al. (US Patent No. 5,549,892) and Smith et al. (US Patent No. 5,939,279). Applicants respectfully disagree, and reiterate the arguments presented in the Reply to Office Action filed on September 23, 2005. Applicants also submit new arguments and the Declaration of Carol Kinoshita Under 37 C.F.R. § 1.132 (hereinafter referred to as "the Kinoshita declaration").

As will be discussed in greater detail below, this combination of references does not render the claims *prima facie* obvious because there is no motivation or suggestion to combine these references and no reasonable expectation of success because the combination is inoperable.

Remodeling of a Mature Oligosaccharide is Distinct from Preventing the Processing of a Precursor Oligosaccharide

The Friedman patent describes the comparison of remodeled placental (p-GCB) and remodeled recombinant (r-GCB) forms of GCB. Friedman concludes that remodeled r-GCB is targeted to Kupffer cells better than remodeled p-GCB. Friedman believes that this is the result of several differences between the two remodeled forms. These differences between remodeled p-GCB and remodeled r-GCB include: r-GCB possessing more fucose residues, r-GCB having four complex chains that underwent remodeling while p-GCB had only three complex chains that underwent remodeling, and an amino acid difference at residue 495, with r-GCB having histidine and p-GCB having an arginine residue at that position (see e.g., col. 4 line 49 to col. 5, line 23).

As stated in the Office Action at page 3, Friedman "teach[es] the importance of GCB remodeling for the production of a pharmaceutically effective preparation and the production of

a remodeled recombinant human GCB in CHO cells" (emphasis added). In contrast, the methods recited in the claims are drawn to preventing the processing of precursor oligosaccharides with a class I mannosidase inhibitor. The claimed methods do not pertain to protein remodeling. As described in detail in the Kinoshita Declaration, remodeling is performed on a mature protein (i.e., a protein containing oligosaccharides that have been processed from a precursor form to a mature (e.g., complex) form). In the case of Friedman, the patent describes remodeling complex oligosaccharides.[1] Friedman only describes the remodeling of the placental and recombinant forms of GCB that contain mature oligosaccharides and makes no mention whatsoever about preventing the processing of precursor oligosaccharides to the mature form. Only one example of remodeling is provided in Friedman. As described at col. 6, lines 4-8 of Friedman, "According to the preferred embodiment of the invention, the carbohydrate chains were remodelled during this purification process by sequential treatment with neuraminidase, galactosidase and β-N acetylglucosaminidase." Treatment with these enzymes removes sialic acid moieties, galactose residues, and N-acetylglucosamine residues, respectively. As sialic acid and galactose residues are present on the reducing end of complex mature sugar forms but not on precursor oligosaccharides, it is clear that Friedman discloses remodeling and not inhibiting the processing of a precursor oligosaccharide.

The Office Action at pages 3-4 states that Friedman teaches that remodeling "may be accomplished by several different alternative ways such as utilizing mutant cell lines deficient in certain carbohydrate synthetic pathways (col. 6, lines 1-15)." Elsewhere, Friedman states, "Remodelling may be performed by enzymatic reactions or by other means and may occur during the manufacturing process or as a result of additional steps after manufacturing has been completed" (col. 3, lines 58-61). Neither the language quoted in the Office Action nor the other quoted text offers a teaching or suggestion that the processing of a precursor oligosaccharide should be prevented, e.g., with a class I mannosidase inhibitor such as kifunensine. As further indicated in the Kinoshita Declaration, because the Friedman patent merely compares two remodeled forms of mature GCB (r-GCB and p-GCB), at most, the teachings of the Friedman

[1] The recombinant form of GCB (r-GCB) produced in the CHO cells of Friedman possesses four complex oligosaccharides. The placental form of GCB (p-GCB) which is also analyzed in Friedman, possesses three complex oligosaccharides and one high mannose oligosaccharide.

reference would motivate a skilled practitioner in the field of protein production to remodel r-GCB instead of p-GCB in order to improve targeting efficiency.

Further, in the previous reply, Applicants pointed out that Friedman discloses that r-GCB showed surprisingly improved pharmacokinetics compared to p-GCB and provides that the improved uptake may be due, at least in part, to different carbohydrate structures. Specifically, Friedman emphasizes two carbohydrate differences, both of which highlight the benefits of remodeled complex chains versus, e.g., high mannose content.

Regarding arguments made by the Applicants in their previous Reply, the Office Action states at pages 7-8 that

> These arguments are not persuasive because at the time the invention was made glucocerebrosidase was one of the most expensive enzymes among drugs. This was mostly due to the cost of remodeling. The targeting ability is not the only aspect of the invention that should be considered. Therefore, one of ordinary skill in the art would have been motivated to produce glucocerebrosidase cheaper, even if the resulting product has targeting ability at the acceptable level albeit decreased. Obviously, to remodel glucocerebrosidase using the cell producing it is significantly cheaper than to remodel the carbohydrate structure of the purified glucocerebrosidase using a multistep process. Friedman teaches that the approach used in the patent is one among other possibilities that could lead to the same product whereas said product may have some variations in targeting ability. (emphasis added)

Applicants submit that there is no basis for these allegations. If this quoted passage is referring to the description in Friedman, Applicants point out that its description is misquoted. According to Friedman, the high costs associated with GCB replacement therapy were associated with the placental form of the enzyme and were due to the scarcity of human placenta and the complex protein purification protocol, not with the costs of remodeling:

> While enzyme replacement therapy using remodelled p-GCR has been shown to be effective in treating patients, it is an expensive form of therapy and places a heavy economic burden on the health care system. The high cost of p-GCR results from the scarcity of the human placental tissue from which it is derived, a complex purification protocol, and the relatively large amounts of the therapeutic required for existing treatments. (Friedman col. 1, lines 48-55; emphasis added)

Moreover, Applicants note that the Examiner's alleges motivation is flawed. The Examiner states that one would be motivated to make GCB "cheaper, even if the resulting product has [decreased] targeting ability." This allegation does not take into consideration that decreased targeting ability may result in higher doses being required and thus, greater production being needed. Therefore, this would not suggest that a drug with decreased targeting ability would be cheaper.

The Smith patent teaches that Enterobacter bacteria bind to high mannose structures on the surface of mammalian cells as a first step in infection. To confirm their model, Smith grew mammalian (HT-29) cells in the presence of kifunensine, a class-I mannosidase inhibitor, so that proteins on the cell surface would have high mannose structures (see e.g., Example 8 beginning at column 8). This resulted in increased bacterial adherence to the cell surfaces.

The Office Action states at page 4 that:

> Smith et al. (US Patent 5,939,279) teach that growing eukaryotic cells in the presence of inhibitors of glycoprotein processing can alter N-linked oligosaccharides. They teach that two such inhibitors, deoxymannojirimycin and kifunensine, inhibit α-1, 2 mannosidases that trim mannoses from $Man_9(GlcAc)_2$ (column 8, lines 4-15). They teach the method of preparing high mannose $Man_9(GlcAc)_2$ glycoproteins by treating human HT-29 cells with mannosidase I inhibitors, deoxymannojirimycin or kifunensine (columns 7-8, column 9, claim 8). With regard to claims 109 and 110, Smith et al. teach the required range of the kifunensine concentration (column 8, lines 24 and 25). With regard to claims 111-114, Smith et al. teach the required range of the swainsonine concentration (column 8, line 26). Therefore, *Smith et al. teach a general method of altering oligosaccharides attached to protein moiety in glycoproteins by growing human cells in the presence of inhibitors of glycoprotein processing*. They teach that the treatment of human HT-29 cells with kifunensine results in glycoproteins comprising $Man_9(GlcAc)_2$. One of such glycoproteins present in HT-29 cells is GCB. (emphasis in original)

The Office Action is over-stating the teachings of Smith. The Smith patent does not teach "the method of preparing high mannose $Man_9(GlcAc)_2$ glycoproteins by treating human HT-29 cells with mannosidase I inhibitors, deoxymannojirimycin or kifunensine" or "a general method of altering oligosaccharides attached to protein moiety in glycoproteins by growing human cells in the presence of inhibitors of glycoprotein processing." Rather, Smith teaches that

the use of kifunensine or deoxymannojirimycin increases the number of $Man_9(GlcAc)_2$ structures on proteins found on the surface of cells, not on all glycoproteins such as GCB. Taken in context, the pertinent portion of Smith recites:

> Two such compounds, deoxymannojirimycin and kifunensine, inhibit the α-mannosidases that trim the first 4 mannoses from the $Man_9(GlcAc)_2$ oligosaccharide (7), whereas castanospermine prevents the removal of the glucose residues (5,31). As a result, mannosidase I inhibitors increase the number (i.e., the amount) of $Man_9(GlcAc)_2$ structures present at the cell surface by effectively preventing their conversion to complex (and hybrid) structures. These inhibitors thus provide valuable tools to determine the in vivo role of high-mannose structures on the adhesion of Enterobacter cloacae. (col. 8, lines 8-17; emphasis added)

Thus, Smith offers no teaching or suggestion that kifunensine or another class I mannosidase inhibitor could or should be used to prevent the processing of oligosaccharides on proteins which are not present on the cell surface, such as GCB. Indeed, GCB localizes to the lysosomes or is secreted from cells.

Finally, the Smith patent is completely unrelated to the field of protein production. The studies in Smith pertain to elucidating the mechanism by which bacteria adhere to the surface of mammalian cells in order to infect the cells. Also, as stated in the Kinoshita Declaration, "Because this reference is concerned with bacterial infections and has nothing to do with the production of mammalian proteins, it is my opinion that a skilled practitioner in the field of recombinant protein production would not read this patent as part of their professional activities" (par. 6). Thus, Applicants submit that the Smith patent is non-analogous art that cannot be relied on for an obviousness rejection because it is not in the field of Applicants' endeavor and it is not reasonably pertinent to the particular problem with which the invention is concerned (MPEP 2141.01(a)).

The Office Action states at pages 4-5:

> Therefore, at the time the invention was made, the importance of remodeling GCB to produce hmGCB has been acknowledged. Remodeling by growing eukaryotic cells in the presence of inhibitors of glycoprotein processing has been known. The use of mannosidase inhibitors, such as kifunensine, as a tool for such remodeling to obtain $Man_9(GlcAc)_2$ oligosaccharide was known. (emphasis added)

Applicants again point out that the remodeling of a mature oligosaccharide described in Friedman is not the same procedure as preventing the processing of a precursor oligosaccharide to a mature form. Remodeling occurs after a mature oligosaccharide has been synthesized. Further, and as will be discussed in detail below, class I mannosidase inhibitors cannot be used in the methods of Friedman to effect oligosaccharide remodeling. As described in the Kinoshita Declaration (see e.g., par. 5), remodeling involves the use of enzymes to remove residues from an oligosaccharide; in contrast, mannosidase inhibitors prevent the removal of residues that occurs in processing a precursor oligosaccharide to a mature form.

The Office Action further states at page 5:

> It would have been obvious to one of ordinary skill in the art at the time the invention was made to grow cells that whether recombinantly or naturally express GCB in the presence of mannosidase inhibitors in order to prepare hmGCB. Any human cell such as HT-29, for example, or a mammalian cell transformed with a DNA encoding human GCB such as CHO as taught by Friedman et al. or COS, can be employed.

Applicants respectfully disagree. The combination of Friedman and Smith offer no motivation to combine their teachings. Friedman teaches that mature oligosaccharides on r-GCB or p-GCB can be remodeled. Smith teaches that treatment of HT-29 cells with kifunensine increases the amount of $Man_9(GlcAc)_2$ oligosaccharides on the surface of the cells. There is no suggestion whatsoever to treat cells with a class I mannosiadse inhibitor to prevent the processing of precursor oligosaccharides on proteins, such as GCB, that localize to the lysosome or are secreted.

The Friedman and Smith References Cannot be Combined Because the Combination Would Change the Principles of Operation of Friedman and Would be Inoperable

Further, even if the teachings of Friedman were combined with the teachings of Smith, the combination changes the principle of operation of Friedman and renders it

unsatisfactory for its intended purpose, thus the combination cannot be used to support a *prima facie* case of obviousness (MPEP 2143.01).

To obtain Friedman's remodeled GCB, a mature complex oligosaccharide must be remodeled. If a cell that produces GCB is cultured in the presence of a class I mannosidase inhibitor such as kifunensine, the precursor oligosaccharides on the GCB protein are not processed to their mature forms. As a result, the kifunensine-treated GCB does not possess mature oligosaccharides and thus the oligosaccharides cannot be remodeled (see also Kinoshita Declaration, par. 7). As discussed above, remodeling removes moieties and if precursor processing is prevented (e.g., with a class I mannosidase inhibitor), there are no moieties for the remodeling enzymes to act on. For example, the enzymes used to remodel GCB in Friedman included neuraminidase to remove sialic acids, galactosidase to remove galactose residues, and β-N-acetylglucosaminidase to remove N-acetylglucosamine residues. These enzymes are inoperable on a protein that was synthesized in the presence of kifunensine because oligosaccharides on such proteins do not possess sialic acid moieties, galactose residues, or N-acetylglucosamine residues that are accessible to the enzyme (the N-acetylglucosamine residues present in the pentasaccharide core are not accessible to the enzyme because they are not at the reducing end of the oligosaccharide). Thus, the remodeling methods of Friedman cannot be practiced on proteins that were produced in cells that were treated with kifunensine, or another class I mannosidase inhibitor.

If cells were treated with a class I mannosidase inhibitor such as kifunensine, no mature oligosaccharides would be present on the proteins synthesized by such cells. Thus, proteins with precursor oligosaccharides cannot be remodeled by the methods of Friedman. Because the combination of these references renders the principles of operation of Friedman inoperable, the references cannot render the claims *prima facie* obvious.

No Reasonable Expectation of Success in Combining the References Exists

The Office Action concludes at page 5:

> One of ordinary skill in the ad at the time the invention was made would have been motivated to specifically purify GCB in view of its pharmaceutical importance taught by Friedman et al. The high expectation of success is provided by Smith et al. who teach the requisite step for preparing remodeled glycoproteins. The purification of proteins from the cells is standard in the art and is taught by Friedman et al., for example.

Applicants again disagree. First, just because GCB is important does not render obvious a specific method (e.g., use of class I mannosidase inhibitors) to prepare a specific form of GCB (e.g., a high mannose form). Second, there is no reasonable expectation of success in combining these references because as described above, their combination changes the principles of operation of Friedman. In fact, the combination is inoperable.

Applicants request that the rejection of these claims be withdrawn because a *prima facie* case of obviousness has not been established.

Claims 129, 139-142, 144-164, and 167-171

The Office Action alleges that claims 129, 139-142, 144-164, and 167-171 are obvious in light of Treco et al. (US Patent No. 6,270,989) in combination with Friedman et al. and Smith et al.

The deficiencies of Friedman and Smith are described above. The Treco patent describes methods for producing three proteins: thrombopoietin (TPO), DNase I, and β-interferon by preparing targeting constructs that can be used to introduce a new transcriptional unit into the gene's endogenous locus. The construct can result in increased expression of the gene. This patent concerns increasing the levels of protein expression and thus is not at all related to affecting post-translational modifications of proteins. Indeed, there is no mention at all about post-translational addition of oligosaccharides to proteins. As such, it does not make up for the deficiencies of Friedman and Smith or solve the inoperability of Friedman in combination with Smith.

Applicants request that the rejection of these claims be withdrawn because a *prima facie* case of obviousness has not been established.

CONCLUSION

Applicants submit that the arguments presented herein have overcome the rejections of claims 105, 106, 109-131, 133-142, 144-164, 167-171, 184, and 185 and that these claims are in condition for allowance, which action is expeditiously requested. The Applicants do not concede any positions of the Examiner that are not expressly addressed above.

Enclosed is a $790 check for the RCE and a $1020 check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050, referencing Attorney Docket No. 10278-017001.

Respectfully submitted,

Date: 6/29/06

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